F :NT COOPERATION TREA

From the INTERNATIONAL BUREAU

# PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>18 November 1999 (18.11.99) | |
| **International application No.**<br>PCT/AU99/00267 | **Applicant's or agent's file reference**<br>83966 |
| **International filing date** (day/month/year)<br>09 April 1999 (09.04.99) | **Priority date** (day/month/year)<br>09 April 1998 (09.04.98) |
| **Applicant**<br>SOLOMON, David et al | |

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

04 November 1999 (04.11.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

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Authorized officer

C. Carrié

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# PATENT COOPERATION TREATY
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## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 11 FEB 2000
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| | | |
|---|---|---|
| Applicant's or agent's file reference 83966 | **FOR FURTHER ACTION** | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416). |
| International application No. **PCT/AU 99/00267** | International filing date *(day/month/year)* 9 April 1999 | Priority Date *(day/month/year)* 9 April 1998 |
| International Patent Classification (IPC) or national classification and IPC **Int. Cl.**[7] G01N 27/447, 27/26 | | |
| Applicant GRADIPORE LIMITED et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

| | | |
|---|---|---|
| I | ☒ | Basis of the report |
| II | ☐ | Priority |
| III | ☐ | Non-establishment of opinion with regard to novelty, inventive step and industrial applicability |
| IV | ☒ | Lack of unity of invention |
| V | ☒ | Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement |
| VI | ☐ | Certain documents cited |
| VII | ☐ | Certain defects in the international application |
| VIII | ☐ | Certain observations on the international application |

| | |
|---|---|
| Date of submission of the demand 4 November 1999 | Date of completion of the report 31 January 2000 |
| Name and mailing address of the IPEA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200, WODEN ACT 2606, AUSTRALIA<br>E-mail address: pct@ipaustralia.gov.au<br>Facsimile No. (02) 6285 3929 | Authorized Officer<br>**STEPHEN CLARK**<br>Telephone No. (02) 6283 2164 |

INTERNATIONAL PRELIM[redacted]Y EXAMINATION REPORT

[redacted]rnational application No.
[redacted]T/AU 99/00267

## I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , filed with the letter of .

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , filed with the letter of .

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, was on the basis of the sequence listing:

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

INTERNATIONAL PRELIM[redacted]Y EXAMINATION REPORT

[redacted]ernational application No.
[redacted]T/AU 99/00267

## IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.
- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☐ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- ☐ complied with.
- ☒ not complied with for the following reasons:

Claims 1-8 relate to an apparatus for forming an electrophoresis gel using a container that receives a gel cassette and includes an inlet port and a baffle to reduce fluid turbulence.

Claims 9-16 relate to a process for pretreating a gel electrophoresis cassette, preparing initiator and co-initiator solutions, mixing solutions and allowing the solution to polymerise in the cassette.

As the the two sets of claims do not have a common novel feature then they lack unity "a priori".

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- ☒ all parts.
- ☐ the parts relating to claims Nos.

INTERNATIONAL PRELIM[redacted]Y EXAMINATION REPORT

[redacted]ternational application No.
[redacted]T/AU 99/00267

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Claims | 1-16 | **YES** |
| | Claims | | **NO** |
| Inventive step (IS) | Claims | 1-16 | **YES** |
| | Claims | | **NO** |
| Industrial applicability (IA) | Claims | 1-16 | **YES** |
| | Claims | | **NO** |

2. Citations and explanations (Rule 70.7)

NOVELTY (N), INVENTIVE STEP (IS), INDUSTRIAL APPLICABILITY (IA) Claims 1-16

None of the citations alone, or in combination, disclose all of the features of any of the claims.

In particular, the baffle and its positioning for the gel forming apparatus of claims 1-8, and the pretreating and preparing steps of claims 9-16 were not found in any of the citations.

It would not be obvious to a person skilled in the art to combine any of the citations to disclose the features of these claims.

The invention is considered to be industrially applicable.

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 27/447, 27/26

A1

(11) International Publication Number: WO 99/53303

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(30) Priority Data:
PP 2902 9 April 1998 (09.04.98) AU

(71) Applicant *(for all designated States except US)*: GRADIPORE LIMITED [AU/AU]; Riverside Corporate Park, 35–105 Delhi Road, North Ryde, NSW 2113 (AU).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: SOLOMON, David [AU/AU]; 95 Watson Road, Officer, VIC 3809 (AU). CHAN, Grace [AU/AU]; Riverside Corporate Park, 35–105 Delhi Road, North Ryde, NSW 2113 (AU).

(74) Agent: F.B. RICE & CO.; 605 Darling Street, Balmain, NSW 2041 (AU).

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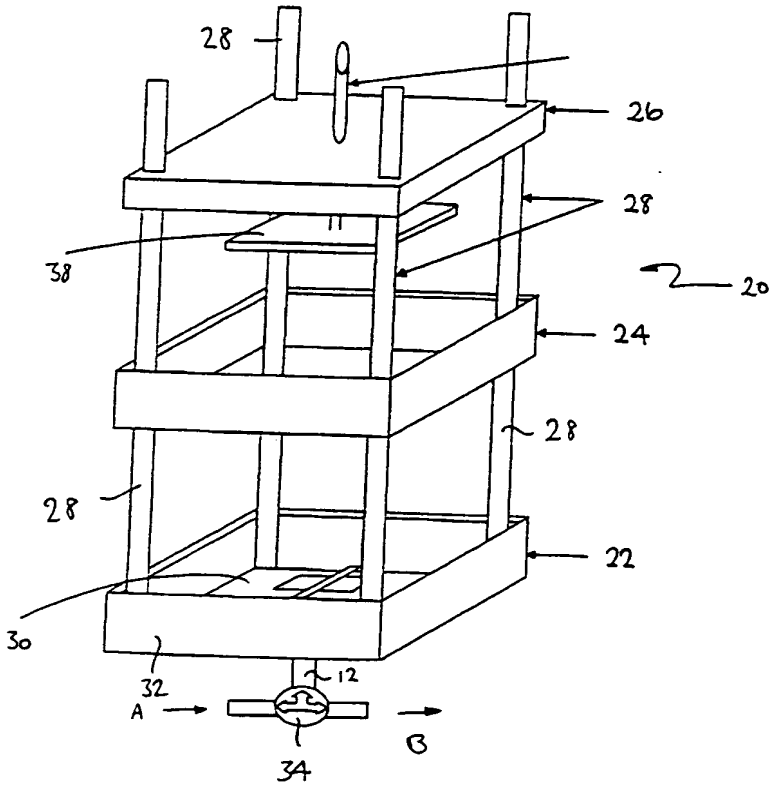
**Published**

*With international search report.*

(54) Title: ELECTROPHORESIS GEL AND GEL-FORMING APPARATUS

(57) Abstract

The apparatus includes a container (20) having a base (22) and sides (32) adapted to receive a plurality of plastic gel cassettes. An inlet port (12) is positioned in the base of the container and in fluid communication with the chamber and a baffle (11) is positioned over the inlet port, such that, in use, when gel forming fluid passes through the inlet port into the chamber, the baffle substantially reduces fluid turbulence and vertical fluid movement in the vicinity of the inlet port during flow of the fluid into the chamber. Pretreatment of the plastic cassette to remove polymerisation inhibitors prior to filling the same with fluid is by exhaustive vacuum treatment, optionally with nitrogen gas purging. This can be achieved conveniently using a vacuum chamber in which one or more plastic cassettes are placed. Optionally the vacuum chamber may be the container in which the cassettes are filled with fluid. No barrier films or chemical scavengers are required.



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[redacted]LD INTELLECTUAL PROPERTY ORGANIZATION[redacted]
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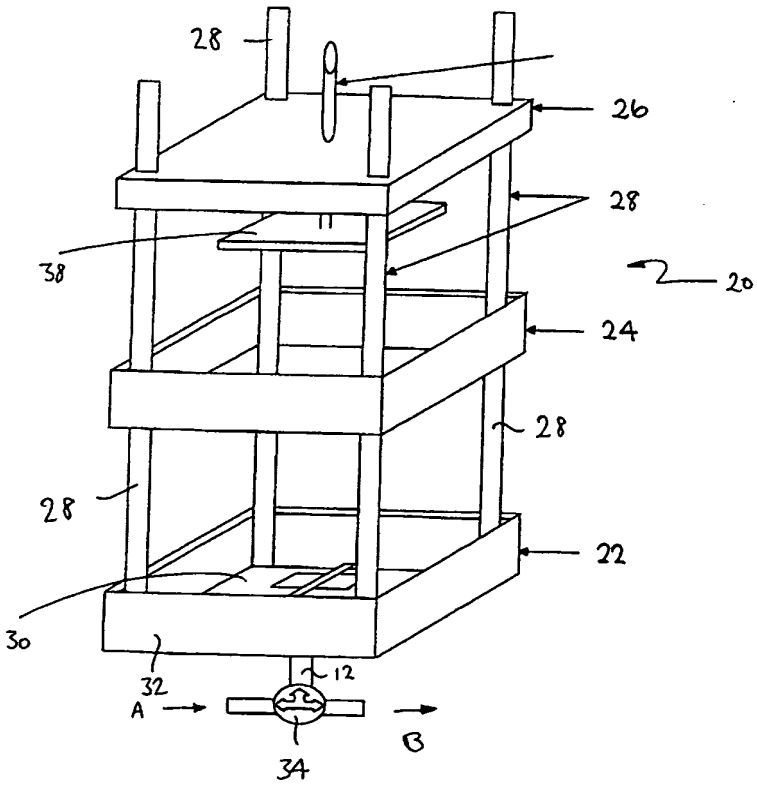

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# ELECTROPHORESIS GEL AND GEL-FORMING APPARATUS

## Technical Field

The present invention relates to electrophoresis gel formation and apparatus suitable for forming gels.

## Background Art

The preparation of polyacrylamide-based matrices for electrophoresis has conventionally involved the aqueous copolymerisation of acrylamide with a crosslinking agent by free radical chemistry. The free radical polymerisation can be initiated by various processes, and once commenced, the polymerisation reaction proceeds until a gel is formed. Gels are often prepared on an individual basis prior to use, and there can be variations between gels that have been cast separately such that comparison between separations using the same gel type are not reliable. Additionally, there has now been a move to the commercial preparation of preformed gels which should have consistent quality and stable physical characteristics between batches.

Traditionally, polyacrylamide gels have been prepared in glass supports. For commercialisation purposes, synthetic electrophoresis gel supports offer a number of advantages over the traditional supports. These include versatility in processing, light weight properties, improved visual appearance, and shatter resistance.

It has long been recognised that the polymerisation and copolymerisation of acrylamide by free radical chemistry is subject to inhibition by a range of compounds. Specifically, oxygen acts to terminate growing polymer chains resulting in longer polymerisation times. Work by other commercial corporations (Daiichi Pure Chemicals and Novel Experimental Technologies) has recognised the impact of such inhibitors and have attempted to address the issues associated with them.

US patent 5350552 (Daiichi Pure Chemicals) describe a batch process in which polyacrylamide gels for electrophoresis are prepared in a container with a low oxygen atmosphere. The batch process involves placing gel supporting plates into the container, in which they are separated by the aid of partition members. The purpose of the partition members is to act as "polymerisation prevention plates" to assist in enhancing and speeding up

the cleaning and processing of the gels with a minimum of gel rejection. The partition members are also used to dissipate the polymerisation exotherm, which is thought to ultimately limit the batch size (up to a maximum of 50 cassettes in the batch). The partition members may be made from a variety of synthetic materials such as polyolefins, polystyrene or fluorinated resins, or from rubber, but should be able to "embrace a large amount of oxygen around its surface with high radical absorptivity." The gel support plates themselves may be made out of glass or plastic.

In US patent 5350552, the inventors recognised the requirement of preparing the gels in a low oxygen environment in order to eliminate the appearance of "flaws or stripes" in the gel. The minimisation of flaws in the gel has traditionally been achieved by the use of an overlay solution, which is employed to prevent the re-absorption of oxygen from the atmosphere by the top portion of the gel. The use of such conditions emphasise that oxygen in the container is a problem, and removal of the oxygen is possible either through application of a vacuum or by displacement with an oxygen-free gas such as nitrogen. After the container is filled with nitrogen, the gel solution is introduced. However, it is not specified whether nitrogen flow is maintained during the polymerisation.

US patent 5685967 (Novel Experimental Technologies) describes a process by which a mould for an electrophoresis gel is coated with barrier films, such as silicone oxide, in order to form a polyacrylamide gel suitable for biological separations. Examples are given in the body of this patent in which various plastic materials, coated and uncoated, were examined for their influence on the gel polymerisation, the resultant physical properties and separation. As an illustration, uncoated SAN (styrene-acrylonitrile) cassettes induced poor polymerisation, and correspondingly, poor electrophoresis results. When the SAN material was coated with PET-SiOx film, the gel quality and performance improved significantly. It was also noted that the oxygen permeability and transmission of the surface in contact with the polymerising solution was an important factor for consideration. This observation is related to an earlier patent specification (WO 90/13020) in which the oxygen permeability of various plastics (PMMA, PET, polystyrene, polycarbonate and polyethylene) and the implication of oxygen in the plastic is discussed.

The present inventors have now obtained improved electrophoresis gels without the aid of barrier films or chemical treatments. Furthermore, large batch production of gels has been achieved by the use of a new gel-forming apparatus.

## Disclosure of Invention

In a first aspect, the present invention consists in an apparatus for forming electrophoresis gels, the apparatus including a container having a base and sides, the container being adapted to receive a plurality of gel cassettes; an inlet port positioned in the base of the container and in fluid communication with the chamber; and a baffle positioned over the inlet port, such that, in use, when fluid passes through the inlet port into the chamber, the baffle substantially reduces fluid turbulence and vertical fluid movement in the vicinity of the inlet port during flow of the fluid into the chamber.

Additionally, other methods of decreasing solution flow turbulence may be used if required, for example a honeycomb or mesh insert or structure may be positioned over the inlet port.

The apparatus may be of any configuration, however, the present inventors have found that a container with a substantially square shaped base is particularly suitable. The inlet port is preferably positioned in the middle of the base of the container with the baffle placed directly over the port. Preferably, the baffle has substantially the same cross-sectional shape as that of the container but of smaller dimension to allow fluid to pass around and over the baffle. The baffle is preferably flat and relatively thin in cross-section to minimise flow turbulence as fluid passes around and over the baffle. The baffle is preferably positioned above the inlet port substantially in the same plane, preferably horizontal, as the base.

Fluid may be moved into the apparatus through the inlet port by any suitable means including pumping or gravity feeding.

In one preferred form, the apparatus is placed in a vacuum chamber to assist in the formation of improved gels according to the present invention.

In a second equally preferred form, the apparatus and vacuum chamber may be combined in one vessel. The vessel may also incorporate heating and cooling means, such that the application and dissipation of heat may be used to advantageously to control the polymerisation. In addition, if

necessary, further engineering refinements for automation of the processes may also be incorporated.

In order to cast a large number of gels in the apparatus, suitable racks which are adapted to hold the cassettes in the correct orientation can be placed in the apparatus.

The apparatus may further include means to control the temperature of the container to assist in the formation of suitable gels. Alternatively, the apparatus may be located in a controlled atmosphere environment.

In a second aspect, the present invention consists of an electrophoresis gel formed by the apparatus according to the first aspect of the present invention.

In a third aspect, the present invention consists of a process of forming an electrophoresis gel in a plastic cassette, the process including the steps of:

(a) pretreating the plastic cassette to substantially remove polymerisation inhibitors present therein;

(b) preparing a monomer solution of acrylamides and treating the monomer solution to substantially remove any oxygen or other gaseous polymerisation inhibitors therefrom;

(c) preparing initiator and co-initiator solutions required to induce polymerisation of the monomer solution, the solutions being treated so as to substantially remove any oxygen or other gaseous polymerisation inhibitors therefrom;

(d) mixing the monomer solution with the initiator and co-initiator solutions to form an initiated monomer solution;

(e) applying the initiated monomer solution to the plastic cassette; and

(f) allowing the initiated monomer solution to polymerise in the plastic cassette.

The cassettes may be manufactured from any suitable synthetic (plastic) material, such as polyesters (PEN, PET, PETG), polyolefins (polyethylene, polypropylene), polystyrene, and any copolymers (SAN), polyacrylics(polyMMA) and any copolymers and vinylidene chloride copolymers. The different materials, however, may require different levels of pretreatment prior to gel formation.

In a preferred embodiment of the third aspect of the present invention, the pretreatment of the plastic cassette is by exhaustive vacuum treatment, optionally with inert gas purging. This can be achieved conveniently using a

vacuum chamber in which one or more plastic cassettes are placed. A vacuum is then applied to the chamber with optional inert gas purging, preferably with nitrogen, if required. The time required to substantially remove polymerisation inhibitors will depend on the type of plastic used. The present inventors have found that pretreatment times from 1 to 12 hours have been particularly successful. It will be appreciated, however, that pretreatment times may vary depending on the type of plastic used and the number of cassettes being pretreated.

In order to remove oxygen and other gaseous polymerisation inhibitors from the various solutions, degassing and optional gas purging have also been found to be particularly suitable. In step (c), one means of ensuring the removal of any oxygen or other gaseous polymerisation inhibitors is to treat water, in which the solutions are made, by degassing and optional gas purging prior to adding the initiator and co-initiators to form the solutions.

In a further preferred form, step (e) applying the initiated monomer solution to the plastic cassette is carried out in the apparatus according to the first aspect of the present invention.

The gels formed may be continuous or gradient gels comprising standard gel forming ingredients having concentrations of monomer and cross-linker as presently used in standard gels known to the art.

In a fourth aspect, the present invention consists in an electrophoresis gel formed by the process according to the third aspect of the present invention.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step or group of elements, integers or steps but not the exclusion of any other element, integer or step or group of elements, integers or steps.

In order that the present invention may be more clearly understood, preferred forms will be described in the following examples with reference to the accompanying drawings.

**Brief Description of Drawings**

Figure 1 is a schematic view of a gel forming tower according to the present invention.

Figure 1a is a diagram showing the positioning of four sets of gel cassettes positioned in the gel forming tower of Figure 1.

Figure 2 is a schematic exploded view of a second apparatus embodying the present invention.

Figure 2a is a plan view from above of a bottom section of the apparatus shown in Figure 2.

Figure 3 shows various gels from positions in a gel forming tower shown in Figure 1a Coomassie blue stained after electrophoresis of protein standards.

Figure 4 shows a temperature vs time plot during acrylamide gel polymerisation of a gel positioned at the centre of the gel forming tower during polymerisation.

Figure 5 shows a temperature vs time plot during acrylamide gel polymerisation of a gel positioned at the edge of the gel forming tower during polymerisation.

Figure 6 shows the stability of various gels cast in the one batch and stored over a 6 month period, Coomassie blue stained after electrophoresis of protein standards.

Figure 7 shows a comparison of two commercial gels with a gel formed by the present invention Coomassie blue stained after electrophoresis of protein standards.

Figure 8 shows a gel stained using silver diamine after electrophoresis of protein standards.

## Modes for Carrying Out the Invention

The present inventors have developed a process in which the inhibiting properties of synthetic electrophoresis gel supports or cassettes, such as polyesters (PEN, PET and PETG), polyacrylics(polyMMA) and any copolymers, polystyrene and its copolymers (SAN) and vinylidene chloride copolymers, can be removed by an exhaustive degassing process. Under going such treatment, the gels according to the present invention prepared in plastic cassettes were equivalent to, or in some instances better than the current commercially available gels.

Furthermore, as a result of this pretreatment, the present inventors have unexpectedly found the polymerisation process required reduced quantities of initiator and co-initiator, and unexpectedly, the polymerisation

exotherm of a gel is much more uniform and controlled. The gels so formed in this controlled exotherm system have greatly enhanced separating properties compared to previous gels made in plastic or in glass cassettes.

The process for the manufacture of improved polyacrylamide gels consists of several components. The components and their role in the process is outlined below:

**Pretreatment Unit (Vacuum chamber set-up)**

This consisted of a high-vacuum chamber, into which high vacuum and an inert oxygen-free gas, preferably nitrogen, was introduced. The gel-forming container which holds the gel cassettes was placed in the chamber and a cycle of vacuum degassing and nitrogen gas purging began (evacuation/nitrogen purge). The pretreatment or degassing cycle may be a single, continuous evacuation, or may be a series of evacuation-purge cycles for a pre-determined length of time.

The pretreatment removes the inhibitors from the plastic cassettes in order to render them suitable for the polymerisation of acrylamide, and different pretreatment times are often required for different plastic cassettes. As an example, cassettes made from the polyesters polyethylene naphthoic acid (PEN) or polyethylene terephthate-co-glycol (PETG) require degassing times of 1 hour and 2 hours respectively, to produce gels of equivalent quality. Cassettes prepared from styrene-acrylonitrile copolymer (SAN) require about 12 hours degassing.

During the polymerisation of acrylamide, it is desirable to maintain an inert atmosphere in order to minimise the incorporation of oxygen and other polymerisation inhibitors into the polymer chain. For example, covalently bonded oxygen becomes a weak link in the polymer backbone as it forms a peroxide bond. Without the use of barrier films or chemical scavengers, polymerisation in synthetic gel supports was not previously thought possible, even within a low oxygen environment.

Additionally, gel polymerisations carried out previously required an organic or aqueous overlay, which can interfere with the polymerisation process. The present invention does not require such measures to obtain suitable gels.

**Gel Forming Container (Tower)**

Previous tower designs have been based on cubic-shaped containers or inverted pyramid designs widening out into cubic shaped area.

A tower for multiple gel casting should satisfy various requirements including having:

minimum hold-up volume

minimum time from the point of initiation to introduction of the solution into the cassettes

non turbulent flow in the tower and cassettes

This has been achieved in the present invention by the use of a tower design which is cubic shaped in which there is a minimum hold-up volume in the area under the level of the plastic cassettes (Figure 1). An inner frame (not shown) on which the cassettes rest may also form part of the tower design, but may not be necessary and its use is dependant upon need and scale. The tower (10) and inner frame may be formed from any material which is not free radical inhibiting and does not interfere with the polymerisation process or solution flow. The tower (10) is preferably made from perspex for ease of processing and visual appeal. It will be appreciated that the tower may also be made of metal including suitable alloys, with or without suitable coatings.

The tower design also encompasses the use of a distributor plate or baffle (12), preferably substantially square in shape with a base (13) and walls (14), of specific dimensions 1/4 to 1/2 way to the cross sectional diameter of the base of the tower, preferably 1/3 of the way, which is placed over the solution inlet port (12). The use of the plate (12) enables a smooth and even flow of the solutions into the tower by dramatically decreasing the vertical velocity of incoming solutions. Thus, particularly for the formation of gradient gels, disturbance to the pre-formed gradient is minimised. This has been evidenced by dye flow tests in towers prepared in accordance with the present invention. The plate (11) may be fixed to the tower (10) by any appropriate manner, for example, by screws to the base (13) of the tower (10), or to a support bar above, or in any other manner which does not impede the solution flow. The height of the plate (11) from the base (13) of the tower is preferably 3 to 10 mm, and more preferably 5 mm. The plate (11) may be made of a material which does not interact with the solutions or interfere with the polymerisation reaction, and examples of such materials are

poly(methyl methacrylate), aluminium, and stainless steel. The plate (11) should preferably be of a minimum thickness, consistent with strength requirements.

**Combined Pretreatment Unit and Gel Forming Container (Reactor Vessel)**

In an alternative arrangement, the pretreatment unit (vacuum chamber set-up and the gel forming container (tower) may be combined to form a single reactor vessel 20 as shown in Figure 2. As shown the reactor vessel is formed in three sections which may be assembled to form a gas tight vessel, although two sections or more than three sections could be provided. The preferred vessel as shown comprises three sections being a base 22, a middle section 24, and a top 26. The sections are square in plan view and are mounted on four support poles 28. The sections may be slid apart to allow access inside the reactor and slid together to form a gas tight container. The base 22 has a square floor 30 and side walls 32 and is used to hold the cassettes and admit solutions. A solution inlet port 12 is provided in the floor 30 and covered with a baffle 11 as in the tower shown in Figure 1. The baffle is mounted on a support beam 16. A valve 34 controls the flow of polymerisation solution A into and waste B out of the reactor. The middle section 24 acts as the body of the reactor and aligns the cassettes. The top section 26 is used to form a vacuum seal by compressing all three sections together. O-rings extend around the edges of the base 22 and top 26 where they face the middle section to form the vacuum seal. A plate is also contained in the top section to assist in maintaining the cassettes in the desired positions and preventing the cassettes from rising when the reactor is filled with fluid. The reactor vessel also incorporates features that facilitate the heating and cooling of the vessel, not shown. The main advantage of this arrangement is the simplification of the manufacturing process, minimising the need for external handling. The reactor vessel is constructed from a material which is suitable for forming a vacuum heating and cooling applications. Suitable materials include aluminium and stainless steel neither of which interfere substantially with the polymerisation reactions. Alternatively, the vessel may be constructed of metals which are coated with substances such as Teflon which do not interfere with the polymerisation reactions.

**Synthetic Electrophoresis Gel Supports (Plastic Cassettes)**

The cassettes may be manufactured from any suitable synthetic material, such as polyesters (PEN, PET, PETG), polyolefins (polyethylene, polypropylene), polyacrylics(polyMMA) and any copolymers, polystyrene and its copolymers (SAN) and vinylidene chloride copolymers. The different materials, however, may require different levels of pretreatment prior to gel formation. Most plastics may be used, even those previously highlighted as polymerisation inhibiting plastics and unsuitable like polystyrene. In addition, some materials may require further treatment before use. For example, the polyester PEN may require, but not necessarily require, a mild, alcoholic caustic etch prior to use. It will be appreciated that any other pretreatment step which renders the cassette material more useful in the formation of electrophoresis gels would be included in the scope of the present invention.

**Scale of Batch Casting**

The batch scale may be readily increased without loss of gel quality. Previously, it has been noted that the batch size for the commercial manufacture of polyacrylamide gels is limited by the resultant polymerisation exotherm. The present inventors have been able to improve on the previous maximum batch size of 50, and were able to produce electrophoresis gels routinely in a batch size of 80. In addition, an increase in scale by 4, with a batch of 320 gels has also been achieved. It is surprising that the exotherm under this large scale casting was controllable and relatively uniform across the batch.

The control over the exotherm was evidenced by temperature sensors present within the gel during manufacture at a central and edge position within the batch.

Correspondingly, the gel quality did not vary across the batch and remained consistent due to the ability to control the exotherm. Experiments performed with other plastics gave similar results for exotherm and other properties, and it appears that batch scale need only be limited by physical practicalities.

**Process of Manufacture**

Initiator Solutions

During the process, there is preferably a sequential addition of the solution components which comprise the monomer mixture. Free radical polymerisation may be initiated by various processes, but from a commercial point of view, the use of a redox system comprising an initiator and a co-initiator is preferred. In contrast to UV photoinitiation or thermal initiation, a redox system is readily adaptable to multiple gel preparations within a batch process. A common redox system is composed of a peroxide based initiator such as ammonium persulphate or potassium persulphate, and a co-initiating agent, which in conjunction with the initiator, is capable of producing free radicals. Examples of co-initiators are N, N, N', N'-tetramethylethylenediamine (TEMED) and 3-dimethylaminopropionitrile (DMAPN).

The process involves the addition of the initiators in a manner such that the minimum holding time is achieved, allowing the solutions to flow into the container and cassettes before the onset of polymerisation. This has the effect of causing:

minimum disturbance of solution flow

minimum disturbance of the desired monomer gradient for gradient gels

minimum batch rejection due to premature polymerisation

The preferred ratio between the initiator and co-initiator components is 1:1. It will be appreciated, however, that other ratios such as 2:1 and 1:2 can be used.

Monomer Solutions

The need to degas the monomer solutions prior to use has long been recognised as a necessary step for the formation of clear and reproducible polyacrylamide gels free of defects. The solutions may be degassed by evacuation using a vacuum pump or water aspirator, or the solutions may be bubbled with an inert gas such as argon, helium or nitrogen, until a very low level of dissolved oxygen is reached.

The use of an improved process incorporating pretreatment of the plastic cassettes, an inert atmosphere for electrophoresis gel formation and degassed solutions enable very low initiator levels to be used when forming the gels in plastic cassettes. Concentrations typically used in acrylamide

polymerisation are in the range of 1 to 10 mM. While initiator levels of less than 1 mM are achievable using glass cassettes, the same low concentrations have previously been found not able to yield good polymerisation in synthetic materials without the use of barrier films or chemical scavengers. With this improved process according to the present invention, initiator concentrations of less than 1 mM are routinely used.

The use of low initiator levels has enabled the production of polyacrylamide gels in plastic cassettes with improved qualities with respect to:

control over the polymerisation exotherm
storage stability
silver staining
protein and other biomolecule separation

The use of high levels of initiator within the process has been shown to cause the monomer solution to polymerise rapidly, producing an uncontrollable exotherm and brittle gels from the evaporation of water from the gel. Additionally, the incorporation of initiator derived fragments (usually $SO_4^-$, if persulphate is used) into the polymer chain introduces charged groups into its structure, which is likely to interfere with the separation of biomolecules, affect the level of sensitivity achieved with silver staining, and influence the matrix stability by catalysing hydrolysis.

Therefore, by minimising the presence of oxygen and other volatile inhibitors, the level of initiator may be adjusted down to very low levels while maintaining satisfactory rates of polymerisation for a commercial scale. While the decrease in oxygen has been recognised to increase the rate of polymerisation, previous practice is not to decrease initiator levels accordingly, as the importance of initiator end groups in the polymer structure has not been recognised.

METHODS

**Plastic Cassette Preparation**

Using SAN cassettes as an example, the pretreatment consisted of subjecting the SAN cassettes to three evacuation/nitrogen purge cycles over a period of 1 hour. Degassing of the cassettes under high vacuum was then left to proceed overnight (12 hours). The cassettes were then subjected to a

further three evacuation/nitrogen purge cycles, and then equilibrated to atmospheric pressure under an atmosphere of nitrogen.

**Solution Preparation**

The required amount of acrylamide and crosslinking agent (generally N, N'-methylene bisacrylamide) to give the desired %T and %C ratio was dissolved in water. To this mixture was added an aliquot of Tris hydrochloride buffer to achieve a final buffer concentration of 0.375M. The solution was then adjusted to pH 8.8 and made up to the final desired volume with water. The monomer solutions were then degassed by bubbling gently with nitrogen gas until less than 1% dissolved oxygen was obtained. Monomer solutions were maintained under an nitrogen atmosphere during gel manufacture.

The water used to prepare the individual components of the redox initiator system, ammonium persulphate and TEMED, was degassed in the same manner. When the desired oxygen level was achieved, the initiator solutions were then prepared.

**Gel Preparation**

With the aid of peristaltic pumps, the individual monomer solutions, the initiator and co-initiator solutions were pumped into the polymerisation apparatus in a sequential manner. The monomer solutions were mixed in-line first, the co-initiator was then added in-line, and finally, the initiator was introduced in-line, prior to reaching the cassettes within the polymerisation apparatus and vacuum chamber. The initiated monomer solution was then pushed up to the required level in the cassettes with the aid of a salt solution. Once in the cassettes, the solution was left to polymerise under an atmosphere of nitrogen, over a period of 2 hours. Once this time had elapsed, the gels were removed from the tower, and placed into a 60°C oven for 1 hour for a post-polymerisation curing step. Alternatively, the gels may be left in the tower and exposed to elevated temperatures *in situ* for curing if required. Once cured, the gels were placed in an 18°C room, and left to cool to room temperature overnight.

EVALUATION OF THE RESULTANT POLYMER MATRICES

Plots were made to show the exotherm is controllable throughout the batch of 320 cassettes (no more than 45°C).

Quality across the batch is maintained

Storage stability trials showed the gels were capable of a shelf-life of at least 6 months at 4°C, while the shelf-life of other commercially available gels in synthetic cassettes was 3 months at 4°C. Similar gels in glass cassettes have been found to have a shelf-life of 30 days at 4°C.

Separation - was at least equivalent or superior to that of other commercial gels.

Silver staining - was shown to be equivalent to, or better than that of other commercial gels

The ability to prepare gels in a variety of different synthetic materials without loss of gel performance was demonstrated.

**SUMMARY**

Polyacrylamide matrices suitable for electrophoresis were prepared by the present inventors in synthetic gel supports without the use of barrier films or chemical scavengers.

The improved method for the preparation of the polyacrylamide gels according to the present invention can be readily scaled up for mass production in a batch process.

The improved batch process incorporates several features which enabled high quality polyacrylamide gels to be prepared in a reproducible manner using a variety of synthetic supports with minimum batch rejection.

The polyacrylamide gels prepared with the improved process had fewer faults in the polymer structure as there was no or minimal incorporation of oxygen, other inhibitors or initiator-derived fragments into the polymer chain.

As a result, the polyacrylamide gels formed using the improved process had a number of improvements over other gels, with respect to:

- control over the polymerisation exotherm
- quality
- storage stability
- separation
- silver staining

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the

invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

## Claims

1. An apparatus for forming electrophoresis gels, the apparatus including:

a container having a base and sides, for receiving a plurality of gel cassettes;

an inlet port positioned in the base of the container and in fluid communication with the chamber; and

a baffle positioned over the inlet port, such that, in use, when fluid passes through the inlet port into the chamber, the baffle substantially reduces fluid turbulence and vertical fluid movement in the vicinity of the inlet port during flow of the fluid into the chamber.

2. An apparatus for forming electrophoresis gels as claimed in claim 1 wherein a mesh or honeycomb structure is positioned over the inlet port below the baffle.

3. An apparatus for forming electrophoresis gels as claimed in claim 1 or claim 2 wherein the base of the apparatus is substantially square and the inlet port is positioned in the middle of the base of the container with the baffle placed directly over the inlet port oriented in substantially the same plane as the base.

4. An apparatus for forming electrophoresis gels as claimed in claim 3 wherein the baffle is substantially square having a side length of 1/2 to 1/4, and preferably about 1/3 of the length of the sides of the square base.

5. An apparatus for forming electrophoresis gels as claimed in any preceding claim wherein the baffle is substantially flat and is thin in cross-section to minimise flow turbulence as fluid passes around and over the baffle and is disposed between 3 to 10mm above the inlet port.

6. An apparatus for forming electrophoresis gels as claimed in any preceding claim wherein the container defines or forms part of a vacuum chamber the arrangement being such that gel cassettes may be degassed in the vacuum chamber and then filled *in situ* with initiated monomer solutions arranged to polymerise in the cassettes.

7. An apparatus for forming electrophoresis gels as claimed in claim 6 wherein the container has at least three sections including, a base section including the inlet and baffle, a mid-section and a top section.

8. An apparatus for forming electrophoresis gels as claimed in any preceding claim wherein the container is formed from aluminium or stainless steel and incorporates heating and cooling means, such that the application

and dissipation of heat may be used to advantageously to control polymerisation in the container.

9. A process of forming an electrophoresis gel in a plastic cassette, the process including the steps of:

(a) pretreating the plastic cassette to substantially remove polymerisation initiators present therein;

(b) preparing a monomer solution of acrylamides and treating the monomer solution to substantially remove any oxygen or other gaseous polymerisation inhibitors therefrom;

(c) preparing initiator and co-initiator solutions required to induce polymerisation of the monomer solution, the solutions being treated so as to substantially remove any oxygen or other gaseous polymerisation inhibitors therefrom;

(d) mixing the monomer solution with the initiator and co-initiator solutions to form an initiated monomer solution;

(e) applying the initiated monomer solution to the plastic cassette; and

(f) allowing the initiated monomer solution to polymerise in the plastic cassette.

10. A process of forming an electrophoresis gel in a plastic cassette as claimed in claim 9 wherein steps (e) and (f) of the process are carried out in an apparatus as claimed in any one of claims 1 to 9.

11. A process of forming an electrophoresis gel in a plastic cassette as claimed in claim 9 wherein steps (a), (e) and (f) of the process are carried out in an apparatus as claimed in any one of claims 6 to 9.

12. A process of forming an electrophoresis gel in a plastic cassette as claimed in any one of claims 9 to 11 wherein the cassettes are made from a synthetic (plastic) material selected from the group comprising:- polyesters (PEN, PET, PETG), polyolefins (polyethylene, polypropylene), polystyrene, and any copolymers (SAN), polyacrylics(polyMMA) and any copolymers and vinylidene chloride copolymers.

13. A process of forming an electrophoresis gel in a plastic cassette as claimed in any one of claims 9 to 12 wherein step (a) includes exhaustive vacuum treatment, optionally with inert gas purging.

14. A process of forming an electrophoresis gel in a plastic cassette as claimed in claim 13 wherein the inert gas is nitrogen.

15. A process of forming an electrophoresis gel in a plastic cassette as claimed in any one of claims 9 to 14 wherein the duration of the pretreatment step (a) is from 1 to 12 hours.

16 A process of forming an electrophoresis gel in a plastic cassette as claimed in any one of claims 9 to 15 wherein steps (e) and (f) are carried out in an inert gas atmosphere.

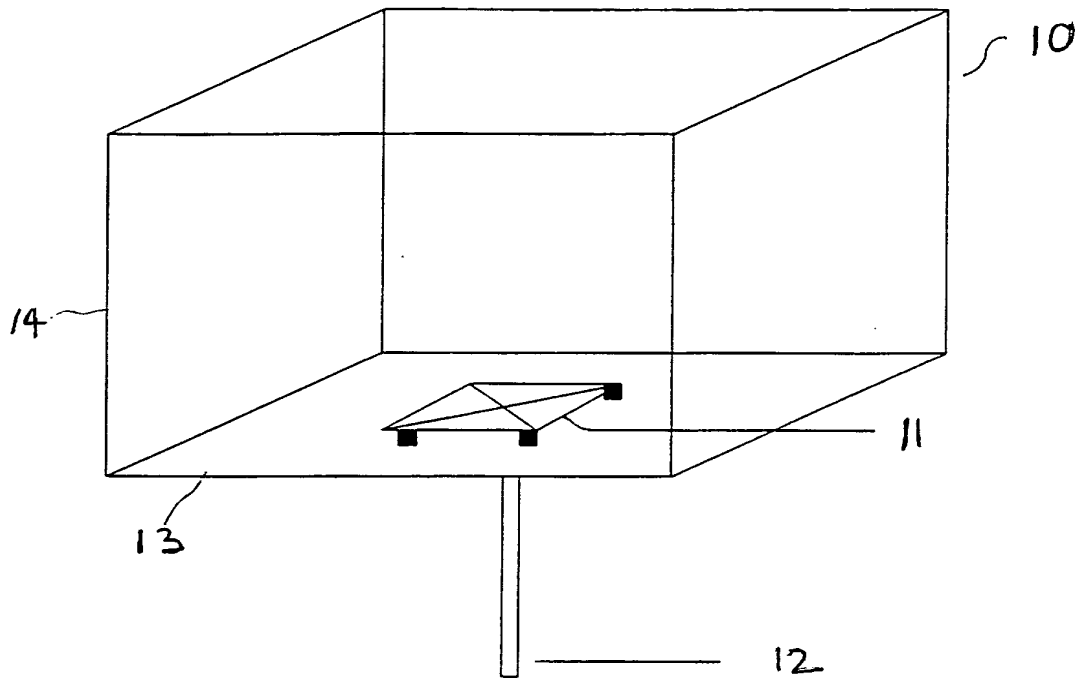


Figure 1

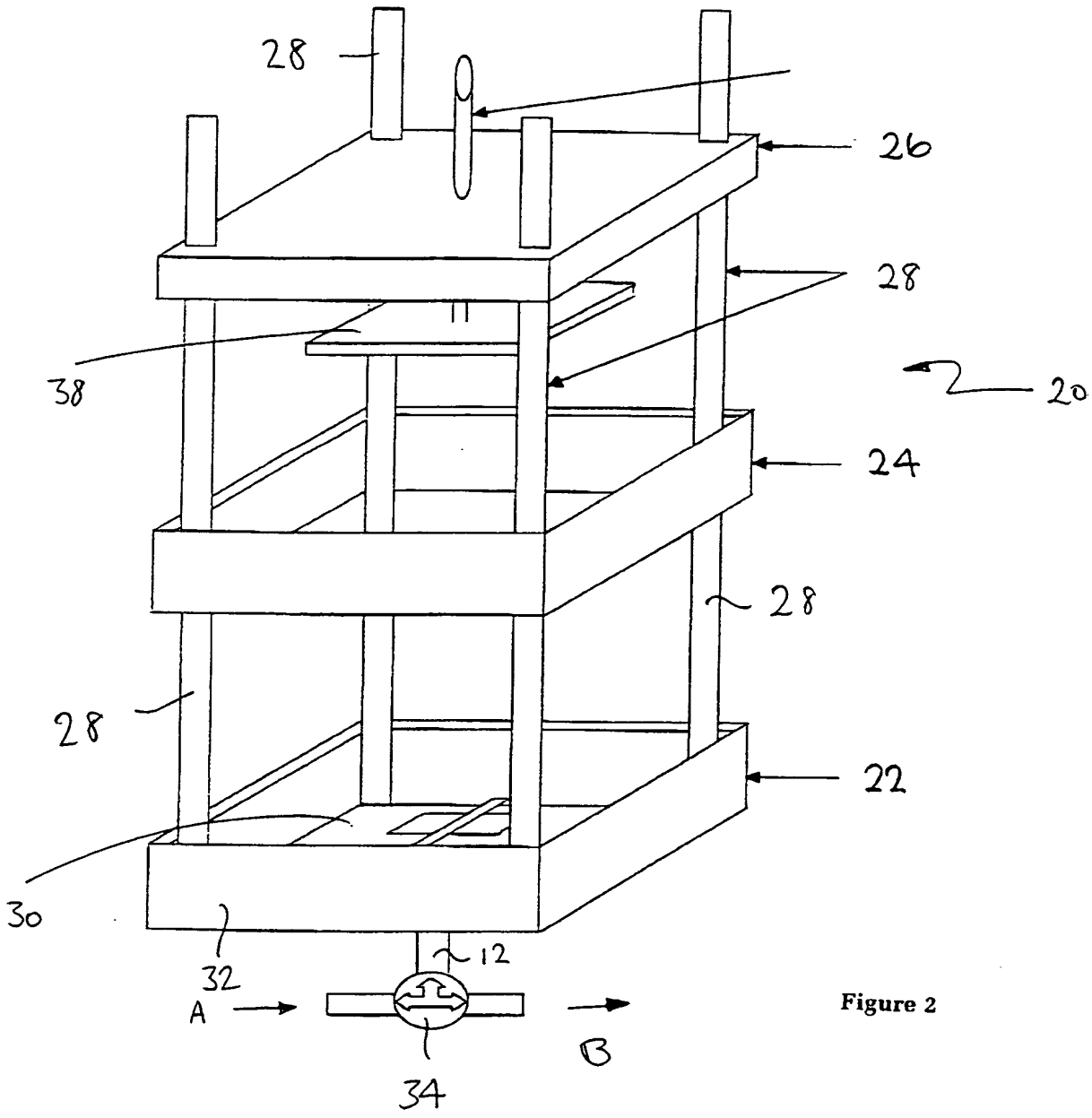


Figure 2

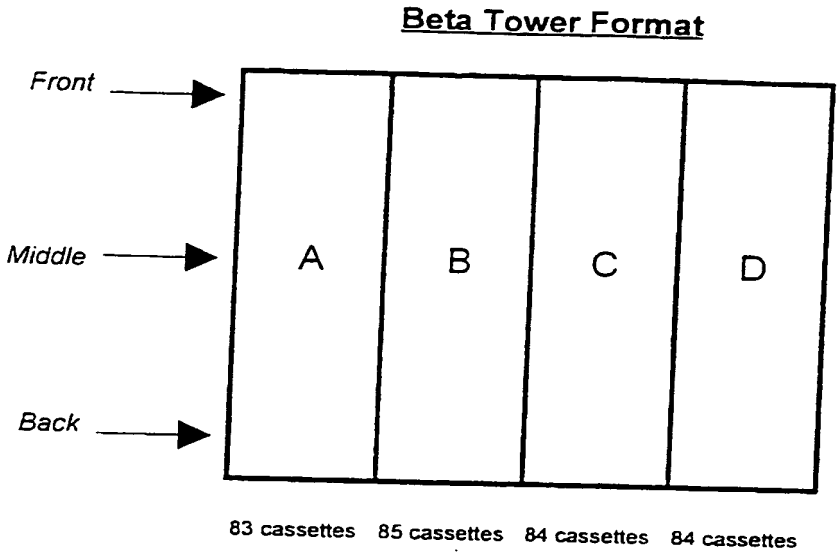


Figure 1a

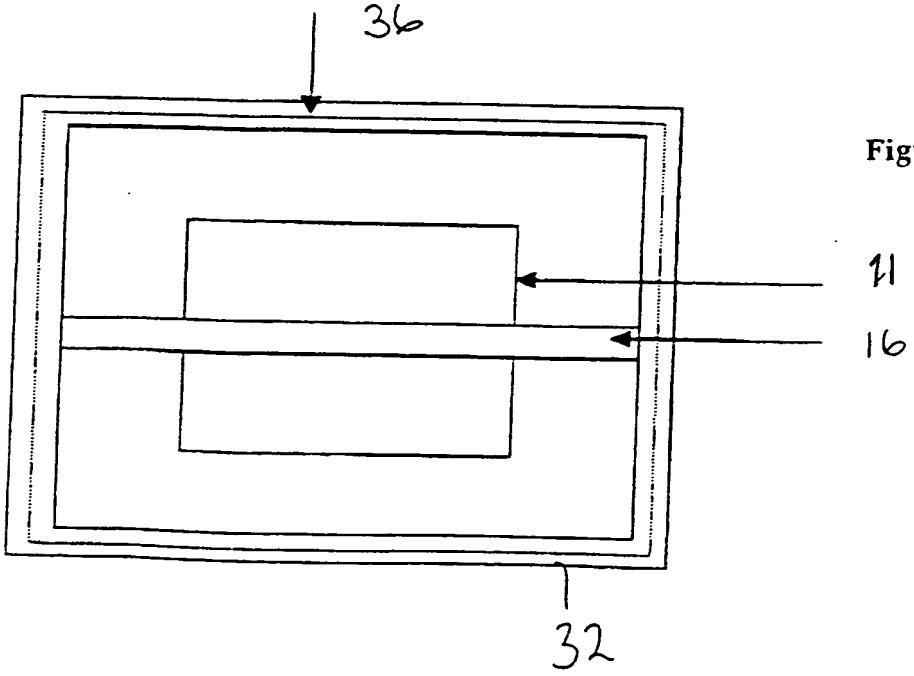


Figure 2a

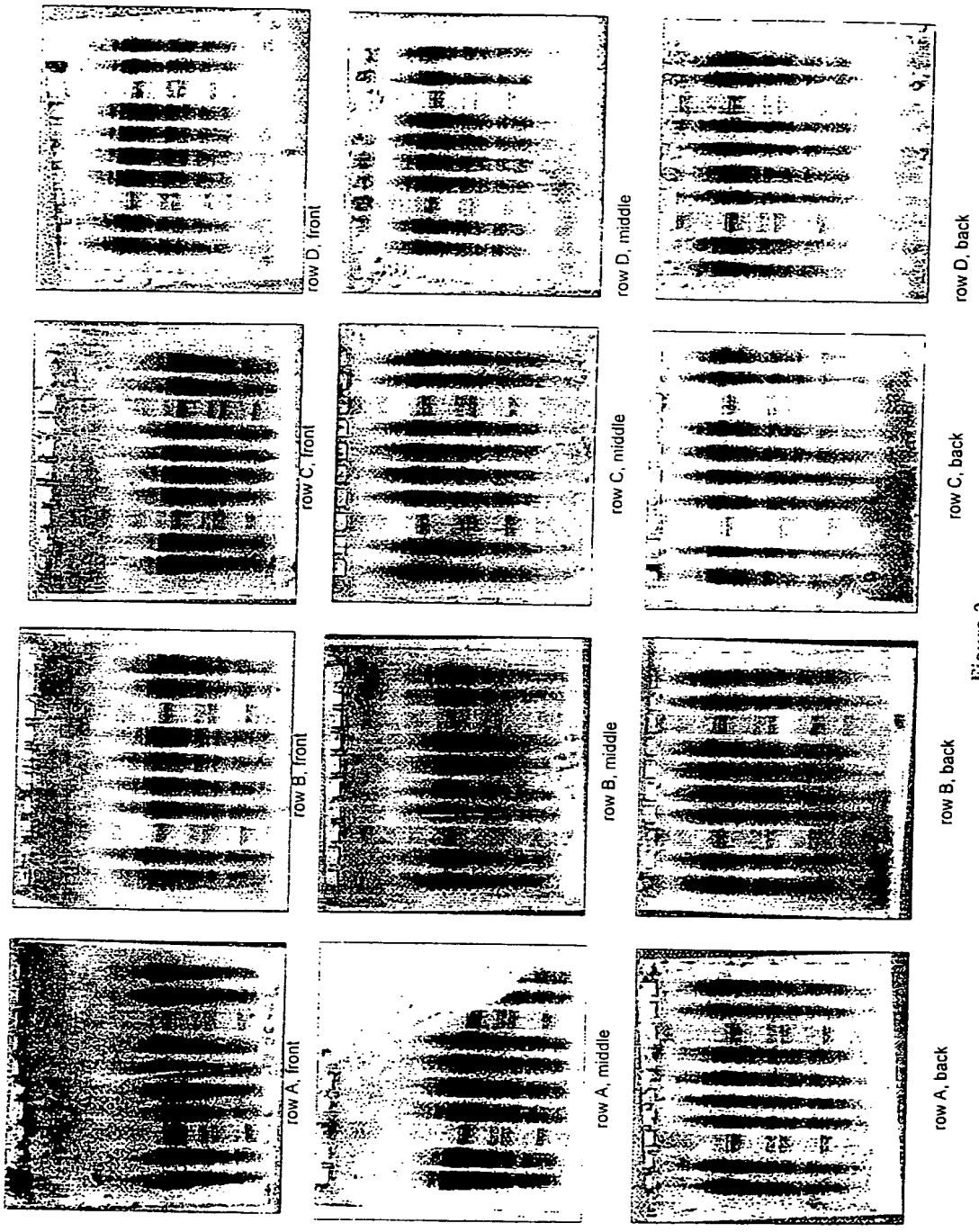
row D, front
row D, middle
row D, back
row C, front
row C, middle
row C, back
row B, front
row B, middle
row B, back
row A, front
row A, middle
row A, back

Figure 3

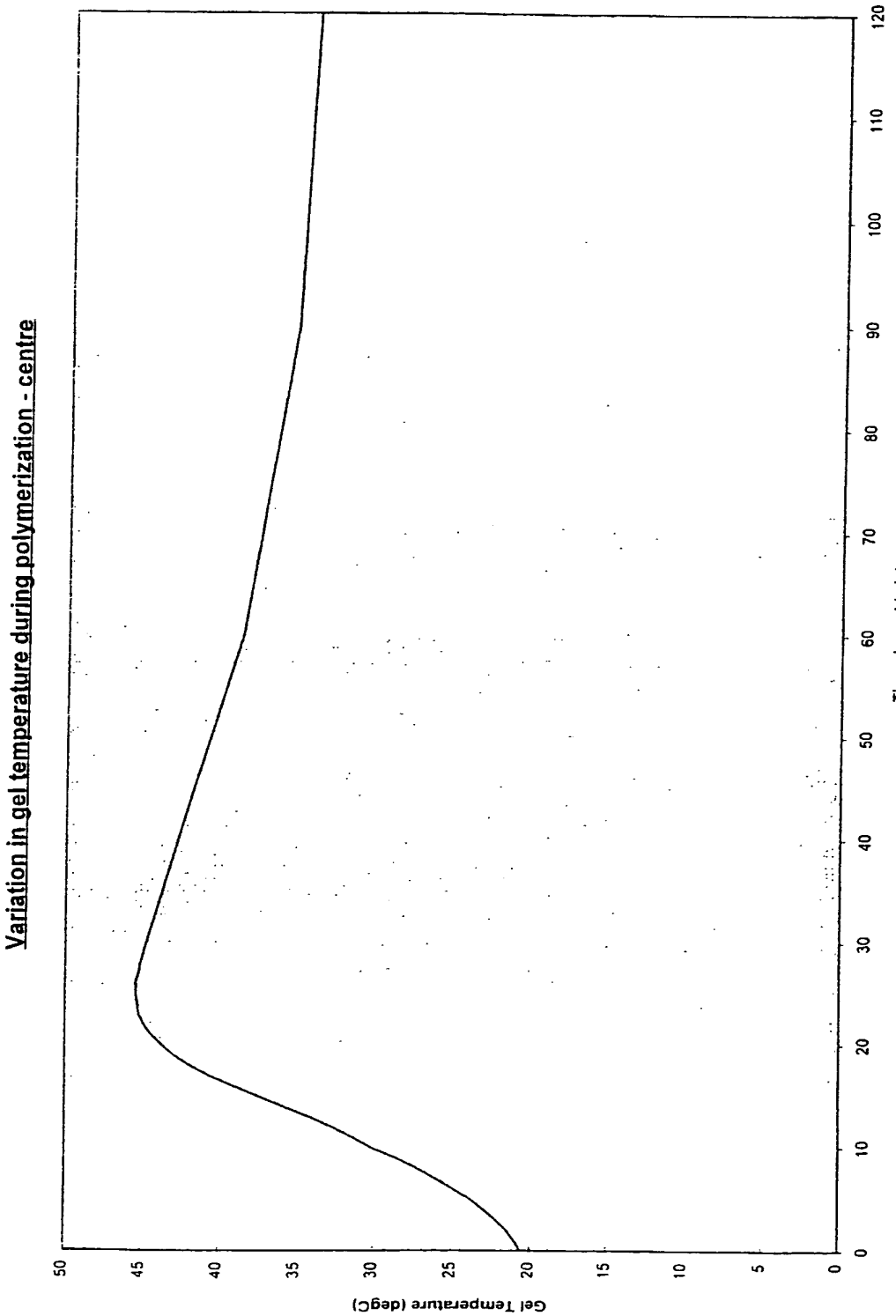
Variation in gel temperature during polymerization - centre
Gel Temperature (degC)
Time elapsed (min)
0
5
10
15
20
25
30
35
40
45
50
0
10
20
30
40
50
60
70
80
90
100
110
120

Figure 4

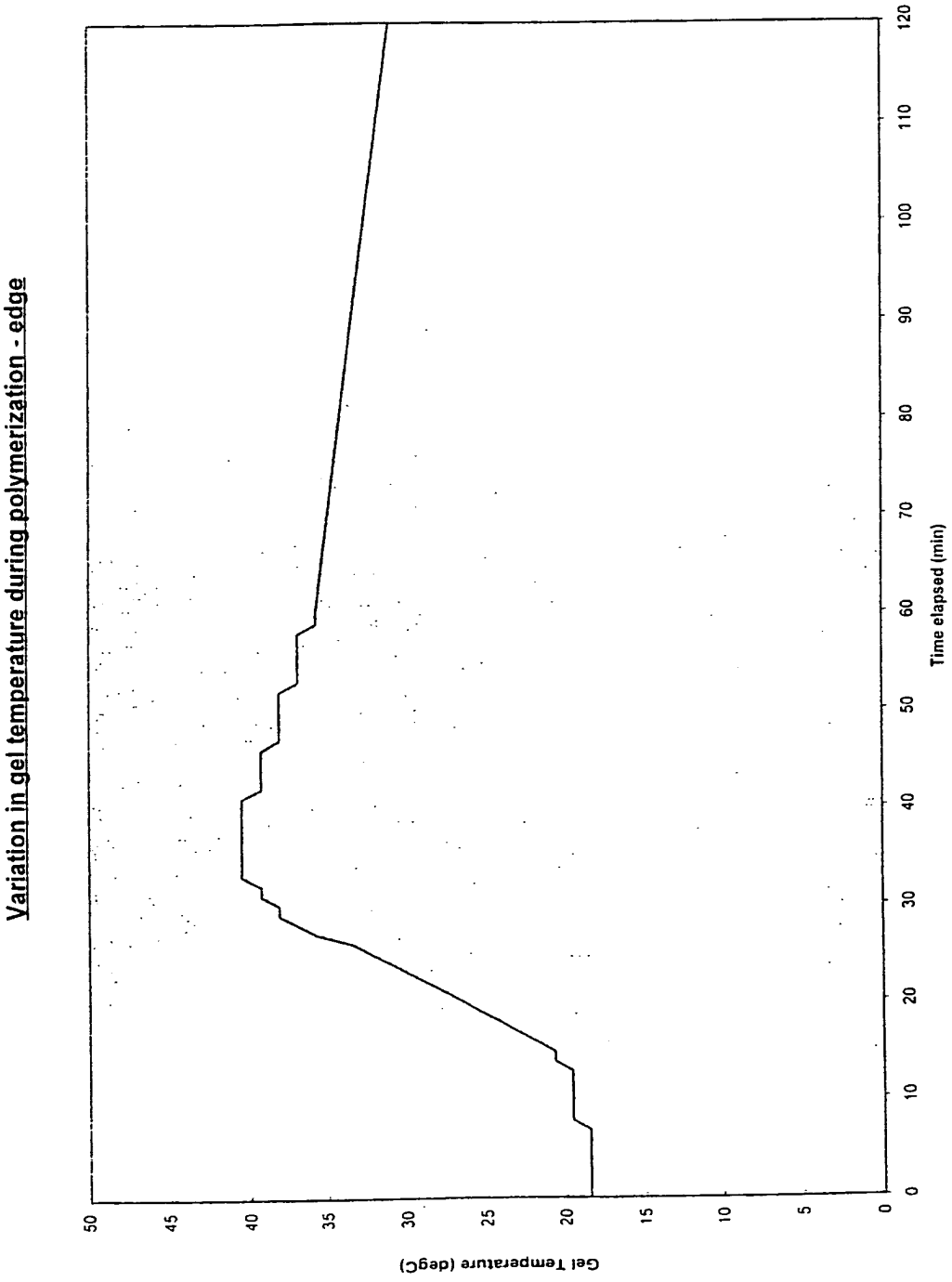
Variation in gel temperature during polymerization - edge
Gel Temperature (degC)
Time elapsed (min)
0
5
10
15
20
25
30
35
40
45
50
0
10
20
30
40
50
60
70
80
90
100
110
120

Figure 5

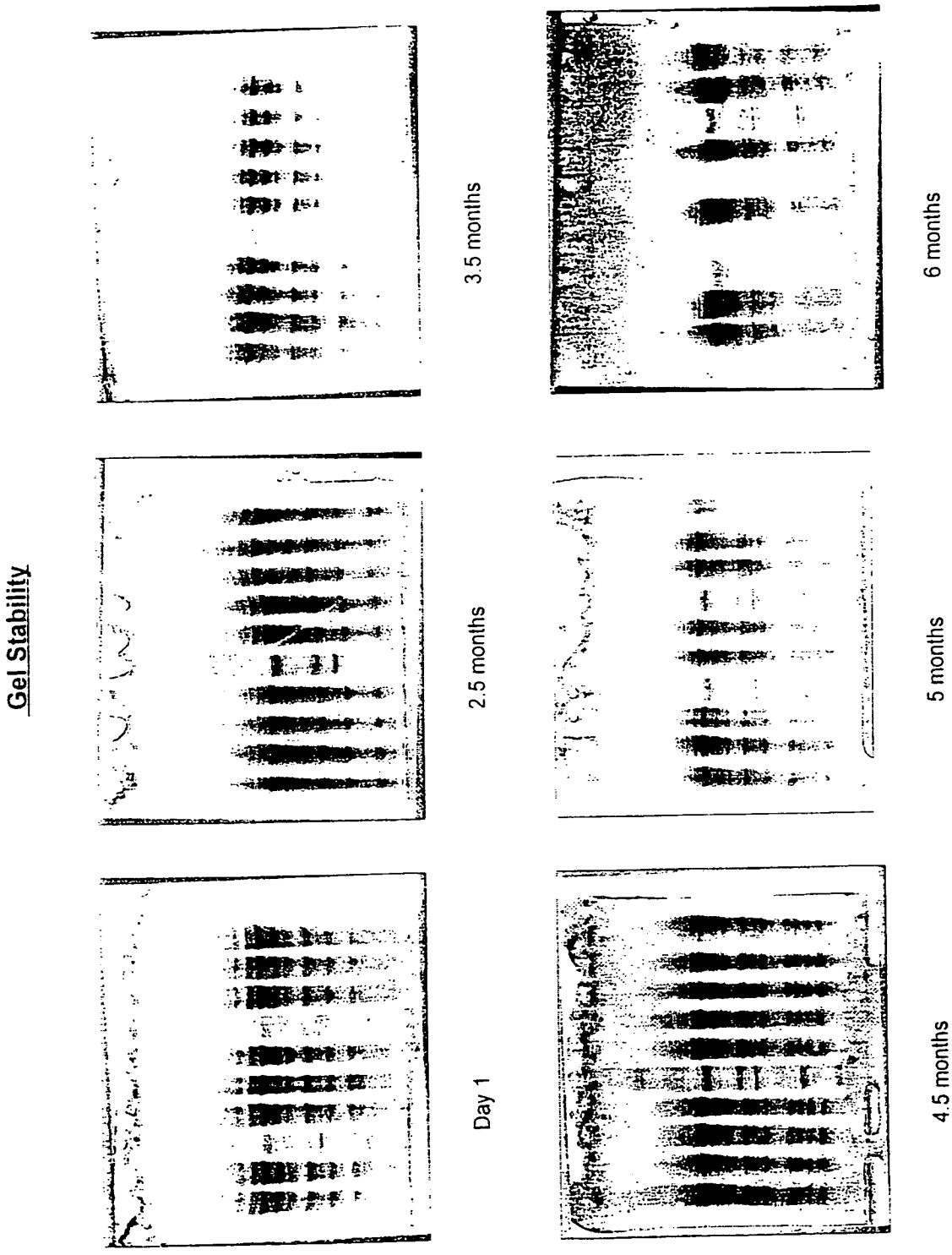
Gel Stability
Day 1
2.5 months
3.5 months
4.5 months
5 months
6 months

Figure 6

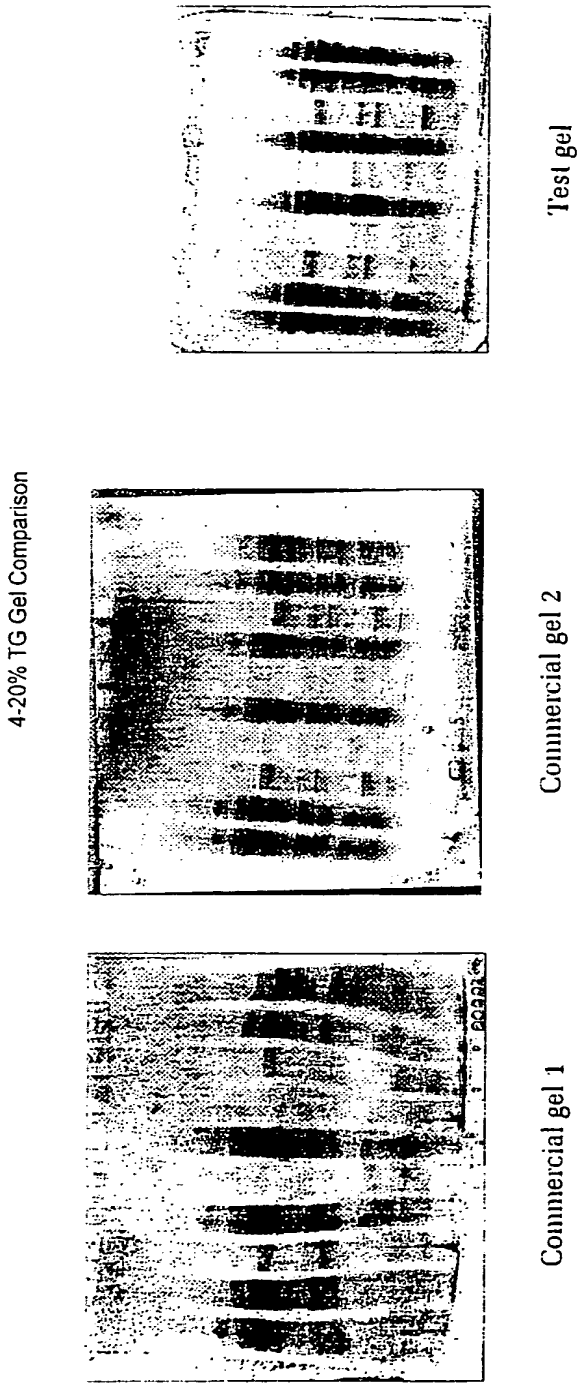
4-20% TG Gel Comparison
Test gel
Commercial gel 2
Commercial gel 1

Figure 7

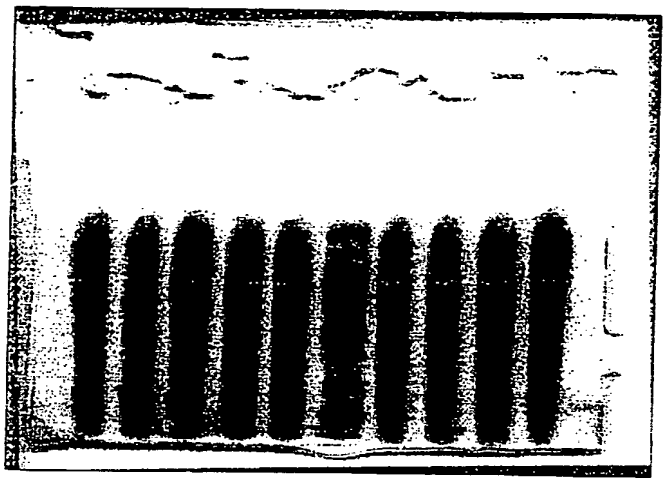

Figure 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00267

**A. CLASSIFICATION OF SUBJECT MATTER**

Int Cl[6]: G01N 27/447, 27/26

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
IPC with keywords

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, JAPIO, USPTO (electrophore:, gel#, cassette#, chamber#, port#, baffle#, mesh:, honeycomb, initiator, pretreatment, oxygen)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | US 5350552 A (EBATA) 27 September 1994<br>See whole doc. | 1-8 |
| A | US 5632877 A (VAN ATTA) 27 May 1997<br>See whole doc. | 1-8 |
| A | Derwent Abstract Accession No. 89-006587/01, Class S03,<br>SU 1404916 A (A MED GENETICS INST) 23 June 1988 | 1-8 |

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 18 May 1999 | 24 MAY 1999 |
| Name and mailing address of the ISA/AU<br>AUSTRALIAN PATENT OFFICE<br>PO BOX 200<br>WODEN ACT 2606<br>AUSTRALIA<br>Facsimile No.: (02) 6285 3929 | Authorized officer<br><br>**STEPHEN CLARK**<br>Telephone No.: (02) 6283 2164 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00267

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | US 5061355 A (ROSE, Jnr.) 29 October 1991<br>See whole doc. | 9-16 |
| A | US 5587061 A (CHEN) 24 December 1996<br>See whole doc. | 9-16 |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU 99/00267

**Box 1 Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

**Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

Claims 1-8 relate to an apparatus for forming an electrophoresis gel using a container that receives a gel cassette and includes an inlet port and a baffle to reduce fluid turbulence.
Claims 9-16 relate to a process for pretreating a gel electrophoresis cassette, preparing initiator and co-initiator solutions, mixing solutions and allowing the solution to polymerise in the cassette.

The above technical features are not regarded as being common to both sets of claims.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
**PCT/AU 99/00267**

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent Document Cited in Search Report | | Patent Family Member | | | | | |
|---|---|---|---|---|---|---|---|
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